iGEM 2014 Measurement Interlab Study Worksheet

To participate in this study, please complete the following worksheet and submit to measurement [AT] igem [DOT] org.

Inter-Lab Study Worksheet:

For each assay that you perform, fill out the following worksheet. Answer each question with enough detail to allow another person to replicate your measurements without needing to ask you any questions. This does not necessarily mean you need to describe everything in detail---for example, if you use a standard assay, you just need to give enough information to allow another person to use that assay in the same way that you did.

Section I: Provenance & Release

1. Who did the actual work to acquire these measurements?

Marlene Sophie Birk, Rik van Rosmalen

2. What other people should be credited for these measurements? (i.e., who would be an author on any resulting publication. For example, your faculty advisor may have helped design the protocols that you ran.)

Wen Ying Wu and Nico Claassen for trouble shooting and general advice. Walter de Koster explaining the measuring device (plate reader)

3. On what dates were the protocols run and the measurements taken? (this will often be a range of dates; make sure you say which data was taken at what times.)

July.2014	Transformation for stock preparation (BBa_E0240, BBa_K516030) from 2014 plates
06.Sept.2014	Transformation for stock preparation (BBa_I20260, BBa_K823005, BBa_K823012) from 2014 plates
07.Sept.2014	Media preparation / Growing of BBa_E0240, BBa_K516030, BBa_I20260, BBa_K823005, BBa_K823012
08.Sept.2014	Miniprep / BioBrick assembly (Digestion/Gel purification/column purification/Ligation)
09.Sept.2014	Transformation of BBa_E0240, BBa_K516030, BBa_I20260, BBa_K823005, BBa_K823012
10.Sept.2014	Colony PCR
11.Sept.2014	BioBrick assembly (Digestion/Gel purification/column purification)
12.Sept.2014	BioBrick assembly (Ligation)

13.Sept.2014	Transformation
14.Sept.2014	Colony PCR \ Sequencing
15.Sept.2014	Transformation for stock preparation of BBa_I20260 from 2011 plate
16.Sept.2014	Growing
18.Sept.2014	Regrow successful transformants for measurement
19.Sept.2014	Measurements of BBa_J23101 + BBa_E0240 in pSB3K3 (2011 Distribution) BBa_J23101 + BBa_E0240 in pSB1C3 BBa_J23115 + BBa_E0240 in pSB1C3
21.Sept.2014	Transformation of 2014 Kit Plate 1-11A (BBa_K516132: J23101 - B0032 - E1010 - B0010 - B0012) Digestion of pSB3K3 backbone from stock
22.Sept.2014	Ligation of pSB3K3 + BBa_K516132. Transformation of ligation mixture into DH5Alfa.
23.Sept.2014	Growing
24.Sept.2014	Miniprep + Sequencing + stock preparation
25.Sept.2014	Overnight growth of RFP devices
26.Sept.2014	Measurements of RFP devices

4. Do all persons involved consent to the inclusion of this data in publications derived from the iGEM interlab study?

Yes

Section II: Protocol

1. What protocol did you use to prepare samples for measurement?

Bacterial specimens and growth media

Escherichia coli strain DH5α was used in this study.

Chloramphenicol, kanamycin and ampicillin were used at a working concentration of 25 µg/mL, 20 µg/mLand 100ug/ml, respectively, in lennox broth (LB) agar plates and liquid LB medium for cultivation

LB liquid media	LB solid media
10 g/L Tryptone	10 g/L Tryptone

5g/L Yeast extract	5g/L Yeast extract
5g/L NaCl	5g/L NaCl
	15g/L Agarose

Growing

Bacterial cultures were grown at 37°C overnight, shaking at 250 rpm in 5ml LB medium containing the appropriate antibiotic.

Miniprep

Plasmids were isolated using GeneJET Plasmid Miniprep Kit (Thermo Scientific). Nuclease free water was used for DNA elution.

BioBrick assembly

Digestion and Ligation was done using New England Biolabs NEB Biobrick Assembly Kit. Digestion was conducted by the restriction enzymes SpeI and PstI to cut the vector and XbaI and PstI to cut the insert. Alkaline Phosphatase (Thermo Scientific) was added to the vector to avoid self-ligation after digestion and incubation time was increased to 1 hour. All parts were gel purified and column purified using NucleoSpin Gel and PCR Clean-up (Macherey-Nagel) and GeneJET PCR Purification Kit (Thermo Scientific). In both cases nuclease-free water was used for DNA elution. Ligation was done at 15°C overnight using T4 DNA Ligase (New England Biolabs). Ligation mixture was prepared with a molar ratio of 3:1.

Transformation

Transformation was done according to the iGEM Standard Transformation Protocol. Commercial DH5-alpha competent E. coli from New England Biolabs were used and the incubation time was reduced to 1 hour and the following heat shock to 30 seconds. For Stock preparation self-made DH5-alpha competent E. coli were used.

Colony PCR

Eight transformants per plate were picked for colony PCR by diluting the colony in 50 µl MQ in PCR tubes and adding 1 µl of this dilution to 19 µl prepared PCR mix. Colony PCR was done using DreamTaq DNA Polymerase PCR protocol (Thermo Scientific) using the forward primer VF2 (BBa_G00100) (5'-tgccacctgacgtctaaga-3') and reverse primer VR (BBa_G00101) (5'-attaccgcctttgagtgag-3') with an annealing temperature of 60°C (15 min 95°C, 30 sec 95°C, 30 sec 60°C, 2 min 72°C, 5 min 72 °C with 35x cycles). PCR products were run on a 1% agarose gel

containing SYBR-Safe for 30 min at 100 V.

2. What sort of instrument did you use to acquire measurements?
    - What is the model and manufacturer?

Synergy Mx Monochromator-Based Multi-Mode Microplate Reader (BioTek)

    - How is it configured for your measurements? (e.g., light filters, illumination, amplification)

GFP: Filter: Gain of 70

RFP: Filter: Gain of 60

3. What protocol did you use to take measurements?

GFP was measured with a gain of 70. Excitation was done at 395/20 nm and emission was measured at 509/20 nm.

RFP was measured with a gain of 60. Excitation was done at 584/13.5 nm and emission was measured at 619/13.5 nm.

Optical density, used as a measure for cell density, was measured at 600 nm.

4. What method is used to determine whether to include or exclude each sample from the data set?

No data was excluded

5. What exactly were the controls that you used?

Negative controls: Water, DH5alpha grown in LB

To correct for background fluorescence: LB-Media

Positive control (GFP): BBa_E0240 under a Lac repressible promoter that has been shown to express GFP

Positive control (RFP): Bba_J04500 + BbaE1010 that was shown to successfully express RFP

6. What quantities were measured? (e.g., red fluorescence, green fluorescence, optical density)

Red fluorescence, Green fluorescence, OD 600

7. How much time did it take to acquire each set of measurements?

20 seconds

8. How much does it cost to acquire a set of measurements?

No significant costs were produced to measure each sample.

9. What are the practical limits on the number or rate of measurements taken with this instrument and protocol?

One plate has 96 wells. Measurement of a whole plate takes about 1 min.

Section III: Measured Quantities

1. For each type of quantity measured (e.g., fluorescence, optical density), report on the following:
2. Units:
    - What are the units of the measurement? (e.g., meters, molecules)
    - What is the equivalent unit expressed as a combination of the seven SI base units? (http://en.wikipedia.org/wiki/SI_base_unit)

green fluorescence	Relative Fluorescence Units (RFU)
red fluorescence	Relative Fluorescence Units (RFU)
OD 600	Relative diffraction

3. Precision:
    - What is the range of possible measured values for this quantity, using your instrument as configured for these measurements? (e.g., a meter stick measures in the range of 0 to 1 meter)

Green/Red fluorescence	0 -100000 RFU
OD 600	0 -1

    - What are the significant figures for these measurement? (e.g., on a meter stick, it is common to measure to the nearest millimeter).

Green/Red fluorescence	1 RFU
OD 600	.001

- Is the precision the same across the entire range? If not, how does it differ?
- How did you determine these answers?

4. Accuracy:
    - When was the instrument last calibrated?
    - How was the instrument calibrated?

Section IV: Measurements

1. For each sample, report:
    - the identity of the sample

First device (GFP): J23101-B0032-E0040-B0015	BBa_I20260 (J23101-B0032-E0040-B0010-B0012) in the pSB3K3 vector. BioBrick Kit location: 2011 Distribution, Plate 2, Well 17F (Use of BBa_I20260 from 2014 distribution was attempted but sequencing could not confirm correct insert)
First device (RFP): J23101-B0032-E1010-B0015	BBa_K516132 in pSB1C3: BioBrick Kit location: 2014 Distribution, Plate 1, Well 11A Transformed into pSB3K3
Second device (GFP): J23101-B0032-E0040-B0015	BBa_K823005 in pSB1C3: BioBrick Kit location: 2014 Distribution, Plate 1, Well 20K BBa_E0240 in pSB1C3: BioBrick Kit location: 2014 Distribution, Plate 2, Well 24B Assembly was confirmed by sequencing
Second device (RFP): J23101-B0032-E1010-B0015	BBa_K823005 in pSB1C3: BioBrick Kit location: 2014 Distribution, Plate 1, Well 20K BBa_E1010 in pSB1C3: BioBrick Kit location: 2014 Distribution, Plate 3, Well 11N Assembly was confirmed by sequencing

Third device (GFP): J23115-B0032-E0040-B0015	BBa_K823012 in pSB1C3: BioBrick Kit location: 2014 Distribution, Plate 1, Well 22I BBa_E0240 in pSB1C3: BioBrick Kit location: 2014 Distribution, Plate 2, Well 24B Assembly was confirmed by sequencing
Third device (RFP): J23115-B0032-E1010-B0015	BBa_K823012 in pSB1C3: BioBrick Kit location: 2014 Distribution, Plate 1, Well 22I BBa_E1010 in pSB1C3: BioBrick Kit location: 2014 Distribution, Plate 3, Well 11N

- each quantity directly measured

GFP Device:	**Measured:**	**Replication:**		
		Replication 1	**Replication 2**	**Replication 3**
H2O	Read 1:600 (OD)	0.044	0.047	0.042
	Read 2:395,509 (Fluorescence)	299	340	357
LB	Read 1:600 (OD)	0.05	0.051	0.043
	Read 2:395,509 (Fluorescence)	22048	22120	22253
DH5alpha	Read 1:600 (OD)	0.776	0.772	0.813
	Read 2:395,509 (Fluorescence)	20119	19881	19955
pLac+GFP	Read 1:600 (OD)	0.623	0.513	0.503
	Read 2:395,509 (Fluorescence)	56757	57141	56455
Device 1	Read 1:600 (OD)	1.056	1.067	1.077
	Read 2:395,509 (Fluorescence)	28525	28535	28520
Device 2	Read 1:600 (OD)	1.099	1.124	1.129
	Read 2:395,509 (Fluorescence)	34700	34662	34279
Device 3	Read 1:600 (OD)	1.104	1.127	1.113
	Read 2:395,509 (Fluorescence)	26911	26522	26777
RFP Device:	**Measured:**	**Replication:**		
		Replication 1	**Replication 2**	**Replication 3**
H2O	Read 1:600 (OD)	0.038	0.038	0.041
	Read 2:584,619 (Fluorescense)	16	44	0
LB	Read 1:600 (OD)	0.041	0.043	0.044

	Read 2:584,619 (Fluorescense)	16	26	8
DH5alpha	Read 1:600 (OD)	1.161	1.175	1.181
	Read 2:584,619 (Fluorescense)	0	10	0
pLac + RFP	Read 1:600 (OD)	1.481	1.518	1.558
	Read 2:584,619 (Fluorescense)	24156	23037	21970
Device 1	Read 1:600 (OD)	1.246	1.29	1.302
	Read 2:584,619 (Fluorescense)	11025	10544	10395
Device 2	Read 1:600 (OD)	1.241	1.29	1.304
	Read 2:584,619 (Fluorescense)	11085	10479	10190
Device 3	Read 1:600 (OD)	1.227	1.263	1.258
	Read 2:584,619 (Fluorescense)	12233	11846	11869

- each quantity derived from measurements (e.g., fluorescence/OD)

Relative GFP/RFP expression:

To obtain relative GFP/RFP expression of each device the average of the relative GFP/RFP expression of the LB-medium was subtracted from the measured relative GFP/RFP expression of each replication of each device. The results were corrected for OD and an average of the three measurements was made.

Device GFP	Relative GFP expression
Device 1	5987
Device 2	11106
Device 3	4125
Device RFP	Relative RFP expression
Device 1	8616
Device 2	9930
Device 3	1195

2. For each group of replicates, report:
   - the identity of samples in the set

First device: GFP: J23101-B0032-E0040-B0010-B0012 RFP: J23101-B0032-E1010-B0010-B0012	Existing device Assembly was confirmed by sequencing.
Second device:	Assembly was confirmed by sequencing

GFP: J23101-B0032-E0040-B0015 RFP: J23101-B0032-E1010-B0015	Assembly was confirmed by sequencing
Third device: GFP: J23115-B0032-E0040-B0015 RFP: J23115-B0032-E1010-B0015	Assembly was confirmed by sequencing Assembly was confirmed by sequencing

- which, if any, of the samples are excluded and why

No samples were excluded

- the mean and standard deviation for each quantity measured or derived

Device	Relative GFP expression (Mean)	Standard deviation	Relative GFP expression (Mean)	Standard deviation
Device 1	5987.588695	50.17686271	8616	217.39
Device 2	11106.74621	277.2242428	8566	301.82
Device 3	4125.025655	179.2630302	9930	146.90